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Developing the Regulatory Utility of the Exposome: Mapping Exposures for Risk Assessment through Lifestage Exposome Snapshots (LEnS)

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Background: Exposome-related efforts aim to document the totality of human exposures across the lifecourse. This field has advanced rapidly in recent years but lacks practical application to risk assessment, particularly for children's health.

Objectives: Our objective was to apply the exposome to children's health risk assessment by introducing the concept of Lifestage Exposome Snapshots (LEnS). Case studies are presented to illustrate the value of the framework.

Discussion: The LEnS framework encourages organization of exposome studies based on windows of susceptibility for particular target organ systems. Such analyses will provide information regarding cumulative impacts during specific critical periods of the life course. A logical extension of this framework is that regulatory standards should analyze exposure information by target organ, rather than for a single chemical only or multiple chemicals grouped solely by mechanism of action.

Conclusions: The LEnS concept is a practical refinement to the exposome that accounts for total exposures during particular windows of susceptibility in target organ systems. Application of the LEnS framework in risk assessment and regulation will improve protection of children's health by enhancing protection of sensitive developing organ systems that are critical for lifelong health and well-being. https://doi.org/10.1289/EHP1250

Introduction

The development of the concept of the exposome (Wild 2005) has provided the field of environmental health with the exciting challenge of identifying and measuring the totality of environmental exposures throughout the life course. Most discussions about the exposome have focused on its potential application in long-term epidemiological studies of cancer or other chronic diseases and have emphasized the need for comprehensive information on lifetime exposures (Wild 2012; Wild et al. 2013). Because continual environmental monitoring remains a challenge, a complete exposome will likely only be developed by combining multiple discrete exposome studies covering different periods of life (Buck Louis et al. 2013; Robinson and Vrijheid 2015; Wild 2012). Current exposome efforts are predominantly focused on this goal of documenting total human exposures across time, and consequently the field has not yet addressed how exposome information can be applied to risk assessment and public health regulations.

Recently, in exploring the value of the exposome in perinatal and reproductive epidemiology, Buck Louis et al. highlighted the importance of evaluating exposures during well-defined critical windows of susceptibility that can be linked to later life disease (Buck Louis et al. 2013; Buck Louis et al. 2017). During these windows, individuals are more vulnerable to the effects of chemical exposures (Buck Louis et al. 2007; Landrigan and Goldman 2011; Selevan et al. 2000). For example, thalidomide causes damage to the embryo when exposure occurs between days 20 and 36 after fertilization, which coincides with an important period of embryonic development; exposures before or after this period have not been found to cause embryotoxicity (Vargesson 2015). Thus, the sum of exposures during such critical periods, rather than just the sum of exposures over the entire lifecourse, may be a particularly significant determinant of disease risk.

To better address the importance of capturing exposures during these critical windows of susceptibility, we introduce a new exposome framework: Lifestage Exposome Snapshots (LEnS). The LEnS approach directs researchers to focus on accounting for all exposures during specific periods of susceptibility for particular target organ systems (Figure 1). Each of these snapshots can capture information on both single and repeated exposures and will provide essential information for epidemiological analysis of both acute and chronic health effects.

This refined concept of the exposome, emphasizing exposures during sensitive periods for particular target organs, provides a more achievable and focused framework for addressing the extraordinary goal of mapping total exposures. The LEnS model also adds a valuable tool for children's health risk assessment and highlights limitations in current regulatory approaches. Below we demonstrate how the LEnS framework can be used in evaluating exposures in the context of children's health through two examples: *a*) within a single regulatory domain for a single chemical class, *b*) across multiple regulatory domains for multiple chemical classes.

Discussion

Implementation across Single Regulatory Domain: Pesticides

Pesticides are governed by three federal statutes. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), all pesticides sold or distributed in the United States must be registered by the Environmental Protection Agency (EPA); this registration process includes an assessment of the risks and benefits of use (FIFRA 1947). The Federal Food, Drug, and Cosmetic Act (FFDCA) requires the U.S. EPA to set pesticide tolerances (the maximum allowable residue level) for all pesticides used in or on food (FFDCA 1938). The Food Quality Protection Act (FQPA) of 1996 amends FIFRA and FFDCA and directs the U.S. EPA to consider the additional susceptibility of infants and children when determining whether the pesticide can be used with a reasonable certainty of no harm. In setting pesticide tolerances, the agency must consider both aggregate exposure (multiple sources

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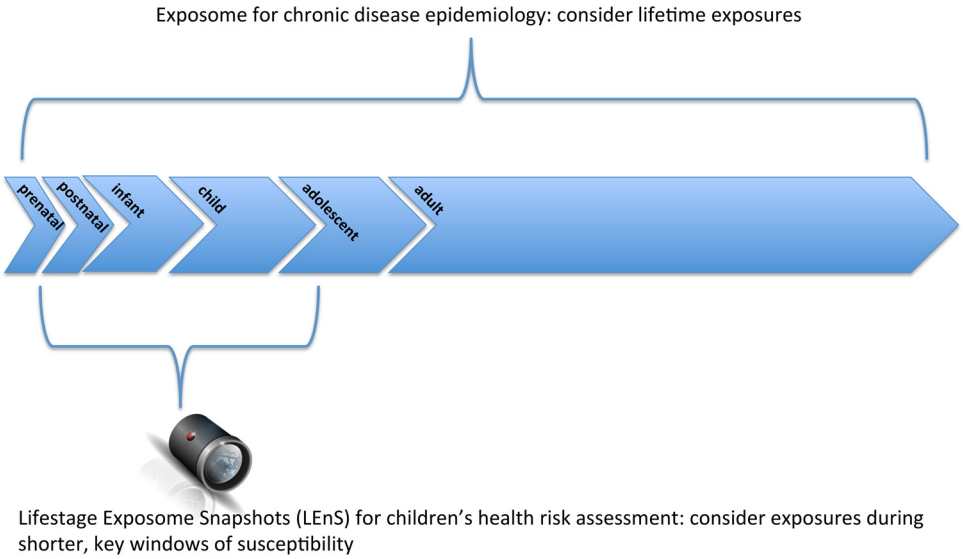

Figure 1. Contrast between previous discussions of the exposome (top), focusing on total lifetime exposures in relation to cancer or other chronic diseases, and new framework for the Lifestage Exposome Snapshots (LEnS) (bottom), based on sensitive periods of development defined by health endpoints of interest. The LEnS approach may be more feasible to implement because the exposure snapshots of interest represent shorter periods of time, compared with the whole lifetime as envisioned in the traditional exposome approach.

of exposure for a single pesticide) and cumulative exposure (exposures to multiple pesticides with common mechanisms of toxicity) (FQPA 1996). [The U.S. EPA's Office of Pesticide Program defines "mechanism of toxicity" as the main steps leading to the toxic effects after the interaction of the pesticide with the biological target. Complete understanding of the biochemical pathway is not necessary; only the key events need to be defined (U.S. EPA 2002). This terminology is similar to the increasingly common mode of action/adverse outcome pathway (MOA/AOP) frameworks, both of which describe the key events leading to the adverse outcome; an AOP also specifically includes the initial molecular initiating event (MIE) (Ankley et al. 2010; Sonich-Mullin et al. 2001; U.S. EPA 2014).]

The agency uses the "risk cup" concept as an analogy for establishing pesticide tolerances. Individual pesticide tolerances must account for all possible exposures to the same pesticide as well as exposures to all other pesticides that act by the common mechanism/MOA/AOP. If these aggregate and cumulative exposures exceed the allocated risk cup, one or more sources of exposure will need to be reduced or eliminated. The U.S. EPA has developed guidance for cumulative risk assessments and categorized five common mechanism groups (CMGs): organophosphates (OPs), *N*-methyl carbamates, triazines, chloroacetanilides, and pyrethrins/pyrethroids (U.S. EPA 2015).

Although the current risk cup framework allows for the combination of chemicals with similar mechanisms/MOAs/AOPs, it does not consider chemicals that act by different mechanisms on similar target organ systems—for example, the effect of exposures to multiple developmentally neurotoxic pesticides. This shortcoming leaves children at risk for health impacts due to cumulative exposures. In order to understand the impact of multiple insults to the same organ system during critical windows of development, a broadened evaluation is essential.

To this end, the LEnS framework can be used to determine the aggregate and cumulative insults to specific target systems during critical windows of susceptibility (Figure 2). As an example of the value of LEnS for regulatory decision making to protect children's health, we present a case study for OP pesticides, which are one of the most commonly used classes of insecticides across the country. OPs are known neurotoxicants, historically thought to exert effects primarily through the phosphorylation of acetycholinesterase (AChE). However, there are multiple additional proposed mechanisms of neurotoxicity, including oxidative stress and interactions with other neuronal proteins (Costa 2006; Lukaszewicz-Hussain 2010; Terry 2012; U.S. EPA 2014). Nevertheless, under existing guidelines from the FQPA, the U.S. EPA assesses cumulative risk to OPs based only on their common potential to inhibit AChE and assumes dose additivity (U.S. EPA 2014). [The U.S. EPA uses dose addition when the effects of chemical combinations can be estimated based on the sum of the relative exposure levels. Key assumptions include noninteraction and toxicologically similarity—either affecting the same target organ or, more specifically, having the same MOA (U.S. EPA 2000)].

The LEnS approach allows for a better understanding of potential effects of multiple exposures on children's health by focusing exposome assessments on critical windows of development for target systems of interest. In considering the neurotoxic OPs, for example, risk assessments should include data from exposome assessments inclusive of key periods of development for the nervous system. Nervous system development begins early in fetal life, with initial neuronal proliferation starting around 5 wk, and includes multiple overlapping processes, including myelination and synapse formation, that continue to occur in early childhood (Figure 2) (Barone et al. 2000; Bernal 2007; Rice and Barone 2000; Rodier 2004). Exposure assessment should account for exposures during these critical pre- and post-natal windows, and chemical kinetics, dynamics, and seasonality of exposures, among other factors, would determine the frequency of sampling (U.S. EPA 1992). Further discussion of study and sampling design is provided below. Each LEnS can provide information about lifestage-specific exposures to target systems of interest (e.g., in Figure 2, neurodevelopment), which can then be evaluated for regulatory purposes.

LEnS analysis I: Mapping pesticide exposures by mechanism. Given the FQPA mandate to assess cumulative exposure to chemicals with common mechanisms, the first way that each pesticide-focused LEnS could be analyzed is by mechanism of toxicity/MOA/AOP. This approach will facilitate the risk assessment process by highlighting co-exposures that should be

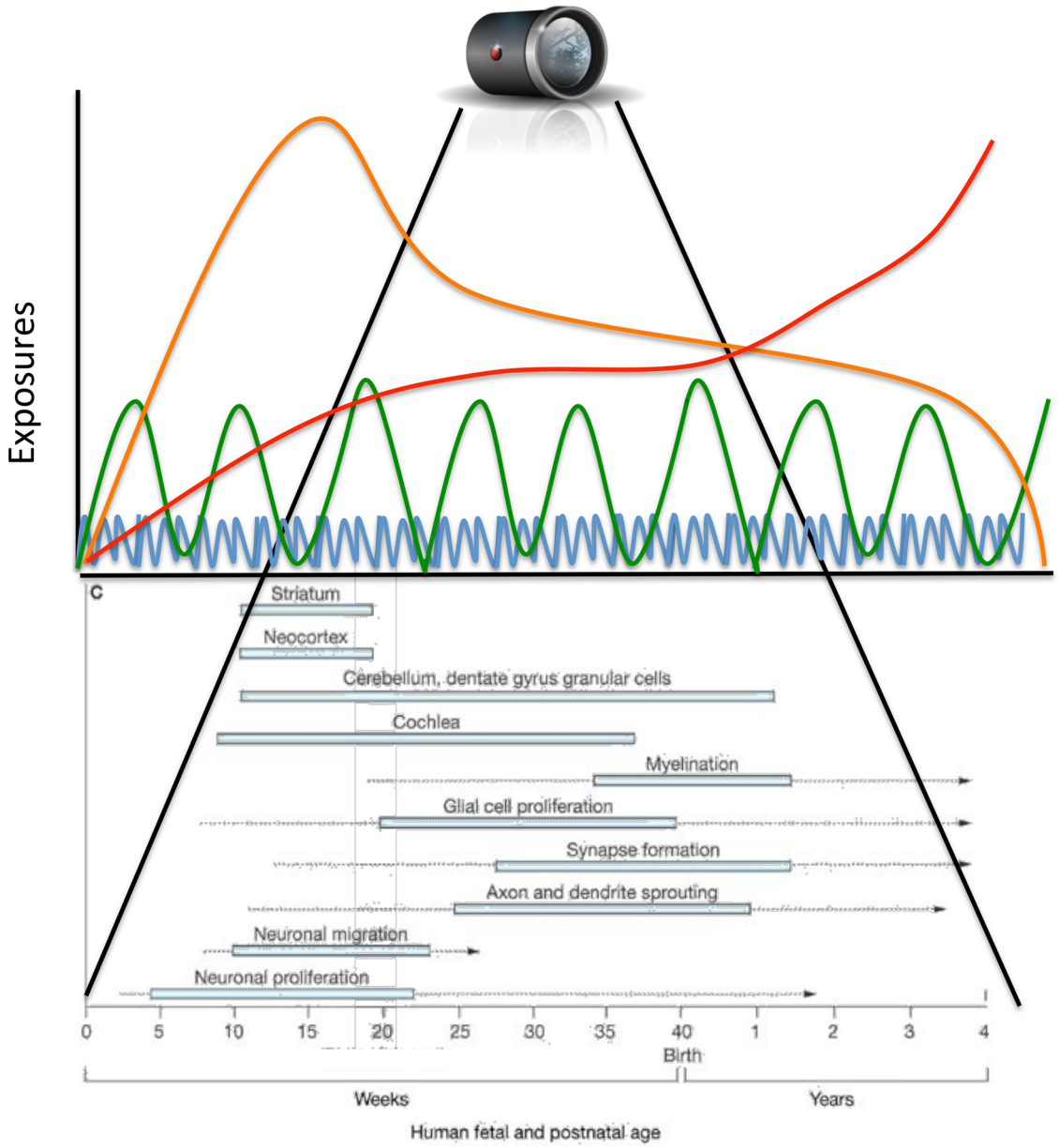

Figure 2. Illustration of multiple environmental exposures during critical periods of brain development. The LEnS approach focuses on lifestage-specific exposures for target systems of interest. Timeline of brain development (bottom) adapted from Bernal (2007), reproduced with permission. Depiction of multiple exposures over time (top) adapted from Robinson and Vrijheid (2015), reproduced with permission.

considered in a common risk cup. For example, total exposures to OPs and other chemicals with common mechanisms should be calculated for each LEnS to determine whether the FQPA standards have been met. This process also complements the recently developed Aggregate Exposure Pathway (AEP) framework, which suggests using target site of an AOP as an organizing principle for exposure analysis (Teeguarden et al. 2016).

Although the presumed primary mechanism for OPs has been categorized, it should be emphasized that describing the mechanism of toxicity/MOA/AOP for the purposes of other pesticide assessments may not require comprehensive understanding of the toxicity pathway (U.S. EPA 2002). Thus, a LEnS analysis for the FQPA should not be delayed because of the absence of complete pathway information.

LEnS analysis II: Mapping pesticide exposures by target system. Although the FQPA does not explicitly require the U.S. EPA to consider combined exposures to different pesticides that act by distinct mechanisms (i.e., independent action) on common target systems or processes, we believe that such analysis follows logically from the agency's mandate to consider the unique vulnerability of infants and children (FQPA 1996). If multiple insults acting by different mechanisms affect common organ systems that are undergoing critical periods of development, they may overwhelm the ability of the system to maintain homeostasis and lead to adverse effects (Cory-Slechta 2005; Rider et al. 2010). Therefore, these simultaneous exposures should be considered together in a "cumulative impacts" assessment based on target organs (Figure 3), as Rider et al. 2010 have proposed.

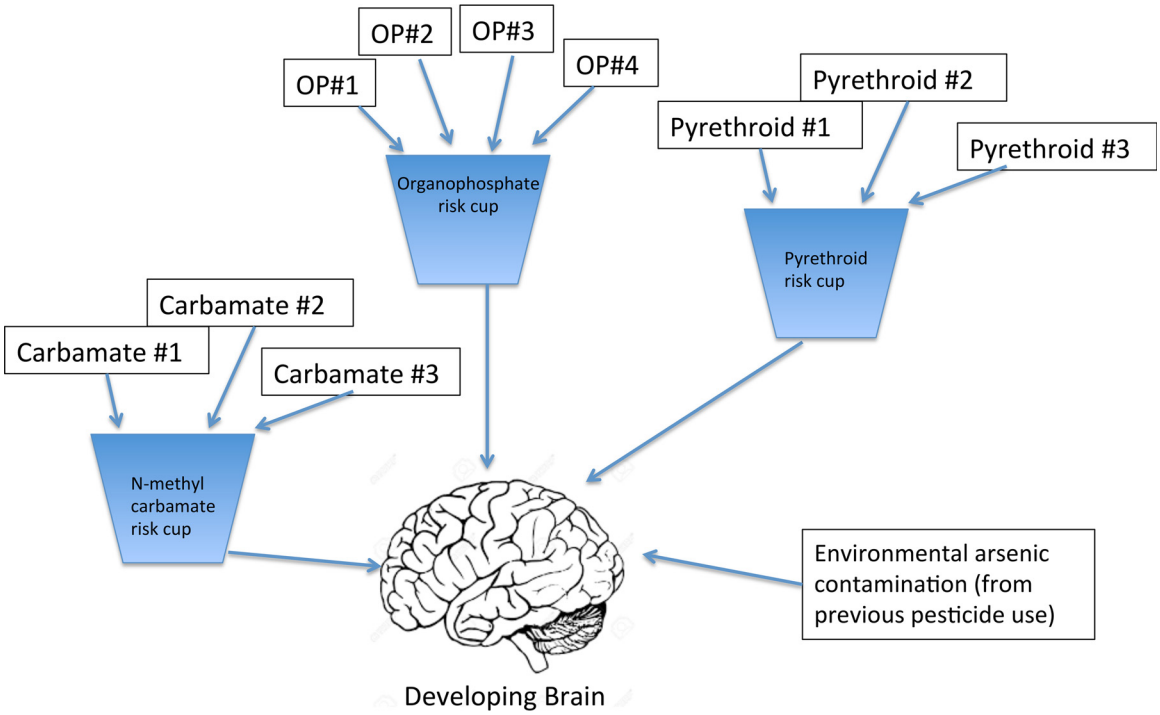

Figure 3. Current pesticide risk assessment requires aggregate and cumulative assessment by mode of action (MOA) and therefore ignores the impact of cumulative exposures to multiple compounds acting by different mechanisms to disrupt the same organ system.

At minimum, the implication of this reasoning is that the U.S. EPA's risk assessment process under the FQPA should not only consider aggregate and cumulative exposures to the neurotoxic OPs but also aggregate and cumulative exposures to all known neurotoxic pesticides during key early life periods. This is especially important as research continues to demonstrate potential alternative mechanisms of toxicity of OPs (Terry 2012; U.S. EPA 2014). Therefore, categorizing OPs only by their potential to inhibit AChE may prove to be too limited for effective risk assessment if the goal is to reduce potential for neurodevelopmental toxicity. In fact, in the recently released chlorpyrifos risk assessment, the agency noted that the data seemed to support "more global alterations in neurobehavioral function" rather than a "specific profile of effects" (i.e., a single mode of action) (U.S. EPA 2014). Recognizing the implications of an overly narrow pesticide evaluation approach based only on mechanism, the European Food Safety Authority (EFSA) has recently proposed to categorize pesticides in cumulative assessment groups (CAGs) based on their "phenomenological" toxic effects, such as impacts to the nervous system or thyroid system (EFSA 2013).

Implementation across Multiple Regulatory Domains: Developmental Neurotoxicants

The second and more far-reaching implication of a LEnS-based analysis is that the U.S. EPA's cumulative risk assessment process should not be restricted based on chemical class. For example, decision making under the FQPA should consider exposures to the neurotoxic OPs in the context of exposures to other known neurotoxicants. The scope of risk assessments should be determined by health outcome of interest, not by chemical use categories (i.e., pesticides vs. consumer product chemicals) or regulatory divisions (i.e., governed by different agencies or statutes) (Evans et al. 2016; Maffini and Neltner 2014). This proposal is aligned with guidance from the National Research Council (NRC) in their report *Phthalates and Cumulative Risk Assessment: The Task Ahead*, which states that cumulative risk assessment should consider chemicals with "common adverse outcomes" rather than only those with common pathways (NRC 2008). Previous experimental evidence also supports this approach (Cory-Slechta 2005; Rider et al. 2010).

Recent publications have classified chemicals that are known or suspected to cause developmental neurotoxicity in humans (Bellinger 2013; Bennett et al. 2016; Giordano and Costa 2012; Grandjean and Landrigan 2014; Heyer and Meredith 2017). These publications illustrate the importance of looking beyond a narrow mechanism of action when evaluating risk. For example, Heyer and Meredith identify 10 stressors representing different chemical classes and mechanisms—including lead, organophosphates, and polychlorinated biphenyls—that may contribute to the development of autism spectrum disorders (Heyer and Meredith 2017). Traditionally, these chemicals would not be evaluated together in a risk assessment. Given their potential for cumulative and joint stress on the nervous system during critical periods of development, however, a more integrative, LEnS-based analysis would provide better protection of children's health.

A LEnS analysis for multiple chemical classes could proceed through two possible paths (Figure 4). In one scenario, problem formulation could begin by identifying the organ system or process of interest (for example, neurodevelopment). Then, based on the timing of development and sensitivity, relevant critical windows of exposure would be identified. Next, exposure assessment would proceed to identify the exposome covering the critical window of neurodevelopment across the population. Finally, a LEnS risk assessment would be conducted based on the cumulative exposures to chemicals targeting the developing nervous system. Alternatively, problem formulation could begin by identifying a chemical of interest. Then, a single chemical exposure assessment would be conducted to identify potential exposures during critical windows of development. Next, one or more critical windows of development would be selected for full exposome

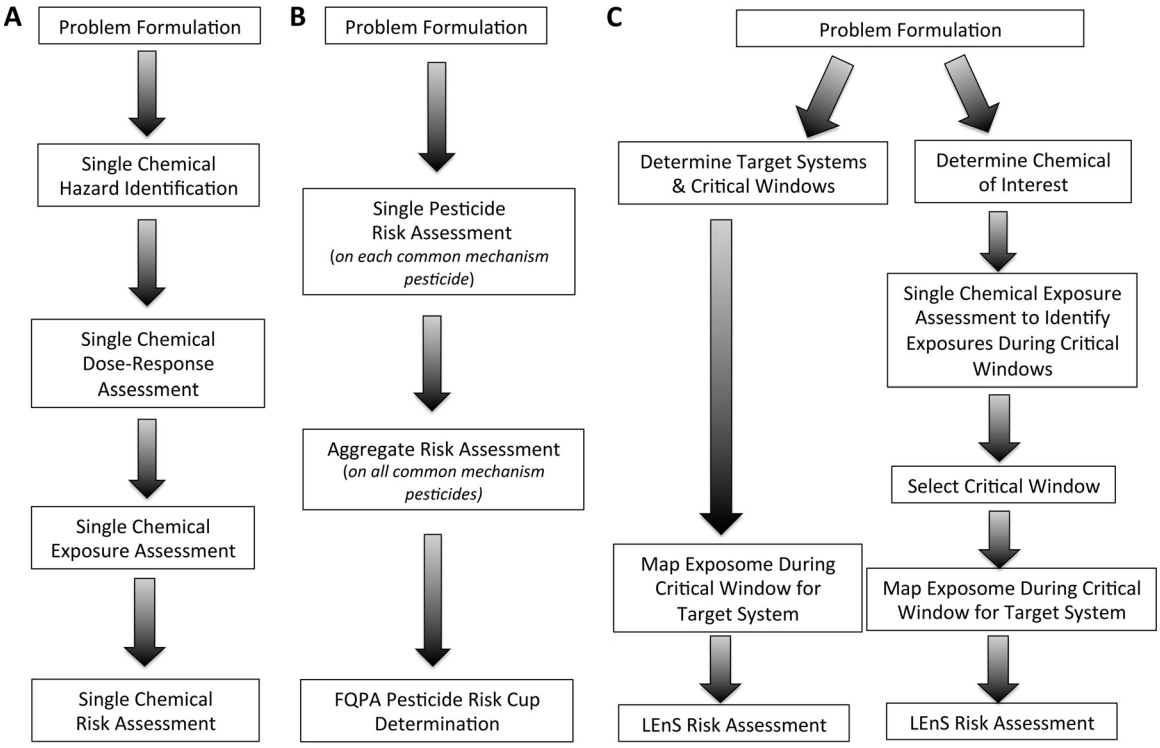

Figure 4. Comparison between (*A*) traditional single chemical risk assessment, (*B*) FQPA risk cup determination, (*C*) LEnS risk assessment based on critical windows.

mapping. This information would be then used in a LEnS risk assessment.

By classifying exposures over defined periods by target system effects, we can better understand total potential hazards to the systems of interest for each LEnS. This information can then be integrated into lifestage-specific risk assessments (U.S. EPA 2006). Combining the effects of chemicals acting by independent mechanisms on similar target organs could proceed via response or effect addition (U.S. EPA 2000, 2007). Evidence suggests that dose addition produces reliable predictions of the effects of mixtures of chemicals acting by diverse mechanisms (Kortenkamp et al. 2012; Rider et al. 2010); however, additional research is needed to further elucidate these patterns. The strengths and weaknesses of existing approaches for cumulative risk assessment have been summarized previously (Reffstrup et al. 2010). The continued generation of this type of data will help to stimulate necessary methodological advancements to address these questions in the coming years.

Study Design and Biomonitoring

Implementation of the LEnS framework can be accelerated by taking advantage of existing population-based studies. Children's cohorts with closely linked exposure measurements covering critical windows are ideal for these efforts (Duncan et al. 2016). Cross-sectional studies would also be appropriate. Population-based sampling surveys, such as the National Health and Nutrition Examination Survey (NHANES), provide information about the representative range of exposures across the general population. If specific information regarding the window of susceptibility during which the exposure occurred is collected in conjunction with exposure levels, these data can be utilized in a LEnS analysis.

Although blood and urine have most commonly been used to assess exposure, alternative emerging methods—using biological samples such as hair, teeth, nails, placental tissue, and meconium—show promise for characterization of past exposures during critical periods (National Academies of Sciences, Engineering, and Medicine 2016; Neri et al. 2006). For example, the half-life of chemical exposures in blood varies, but for some compounds, such as chlorpyrifos, it is estimated to be about 15 h (Griffin et al. 1999). Urine samples can reflect exposure over longer periods of time; however, this method frequently requires collection of 24-h urine excretion (Adibi et al. 2008). Thus, in order to use blood or urine to assess exposure during critical periods of development, it would be necessary to collect multiple precisely timed samples.

Hair, however, can be used to characterize exposure over recent months. For example, a hair sample collected at birth, can allow a researcher to sequentially examine concentrations of chemical exposures or endogenous hormones by trimester of pregnancy (Kirschbaum et al. 2009). By considering between and within person variability, it is possible to use pharmacokinetic and pharmakodynamic modeling to estimate maternal blood and fetal exposures from hair (Bartell et al. 2000; Smith et al. 2014). Teeth can also serve as a retrospective assessment tool for exposure to environmental chemicals (Andra et al. 2015). Because teeth begin to form *in utero*, they create a record of the biological environment throughout gestation and early childhood. Untargeted metabolomic analyses have been able to identify thousands of unique peaks, and targeted follow-up methods have been able to identify multiple phthalates and bisphenol A metabolites in teeth (Andra et al. 2015). This approach provides a unique cumulative method for retrospectively assessing the *in utero* and early childhood exposome.

Nails provide another method for assessing past chemical exposures, with samples usually reflecting exposures approximately 1–2 mo previously (Laohaudomchok et al. 2011). Lastly, placental tissue and meconium are biospecimens that are collected at or around the time of birth to characterize the *in utero*

environment. These biospecimens can be informative for some types of exposures (Green and Marsit 2015; Yusa et al. 2015), but allow for less granularity in timing of exposure compared to hair, teeth, and nails. Advances in the types of biospecimens that can be analyzed for environmental exposures open the door to retrospective exposure assessment and allow for identification of exposures during critical periods of development.

Untargeted exposome analyses can use blood, urine, teeth, hair, or nails to characterize retrospective exposure to a broad range of chemicals. Genome-wide association studies have been conducted for decades. However, to truly uncover the associations between genetics, the environment, and disease, exposure needs to be characterized with the same complexity as genomics (Cui et al. 2016). Liquid chromatography–high-resolution mass spectrometry techniques for untargeted analysis can enable detection of over 10,000 chemicals in biological samples (Uppal et al. 2016a). Precise identification of all of these chemicals is difficult at present; however, as bioinformatics and data extraction algorithms continue to improve (Uppal et al. 2016a), this challenge will be alleviated. Other approaches for circumventing this challenge include focusing on specific biologically relevant pathways, such as inflammation or lipidomics for data analysis (Karnovsky et al. 2012; Zhao et al. 2016).

Overall, the use of novel biomarkers and new untargeted analytical techniques provide the opportunity to retrospectively assess the exposome across the lifecourse. The recently established Children's Health Exposure Analysis Resource (CHEAR) can be utilized to support these efforts: The program offers a network of coordinated approaches for targeted and untargeted analyses relevant for children's health, as well as data standards and a data repository (NIEHS 2016). These developing methods and new infrastructure support will benefit from the new LEnS analytical framework to interpret data in the context of lifestage and children's health.

Challenges to Implementation

There are numerous challenges that researchers would face in implementing the LEnS framework. The first challenge is based on elucidation of the exposome. Two approaches have been proposed for characterizing the exposome, and each has limitations. The bottom-up approach assesses exposure through environmental measurements but would require enormous effort to characterize all relevant environmental inputs. The top-down approach assesses exposure through biological assays but would not provide information on exposure source (Rappaport 2011), which would be critical for LEnS-based policy changes. The top-down approach is also limited based on available technology and the number of chemicals that can feasibly be detected in a biological sample; however, these challenges will be overcome as the technology progresses.

In addition, there are general challenges with regard to the accuracy of biomarkers for the top-down approach, such as detecting chemicals with short half-lives and the question of whether the biomarker accurately reflects exposure during the relevant time period of concern (Braun et al. 2016). Furthermore, traditional targeted analyses often lead to observational bias, in which the chemicals are detected because they are specifically under investigation. Untargeted analyses, however, can help the field move beyond this type of "streetlight effect" (Braun et al. 2016), and recent advancements in the field of metabolomics provide great promise for the identification of the exposome (Jones 2016; Uppal et al. 2016b).

Other difficulties are more specific to the LEnS framework, such as choosing an appropriate biomarker that reflects impacts to the target organ system of interest. Most "omics" techniques utilize blood or urine measurements, but these samples would not necessarily capture a target-organ specific exposome. Emerging techniques, using biological samples such as hair or nails, can provide information on timing of past exposures but likewise may not provide specific information on systems-specific exposures of concern. Therefore, extensive background knowledge would be required to choose an appropriate combination of biological specimens and extrapolate among them to determine an estimated target-organ system exposome. Another challenge is that the exact window of susceptibility has been determined for many but not all biological processes. Information about critical periods is essential to a LEnS analysis.

Sampling would also pose challenges. For example, one measurement of chemicals with changing exposures over time and rapid metabolism would not adequately represent exposure over the duration of the critical period. Thus, chemical kinetics would need to be considered in choosing the appropriate sampling procedures to obtain accurate estimates of exposures over sensitive windows of development. Obtaining high quality data on all relevant chemicals during all critical periods of development seems like near-insurmountable challenge, but rapid advancements in exposure assessment and biological understanding may soon allow this proposal to be realized.

The most significant obstacle, which the authors do not underestimate, is the feasibility of implementing the proposed ideas. Given the enormous effort that is currently needed to conduct single-chemical risk assessments, it may be hard to imagine a system that can efficiently and effectively assess risks from multiple chemicals across different regulatory domains. Yet, there are many examples across science and society of theories that were once far-flung proposals but are now standard or well-accepted ideas. Albert Einstein once said, "To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science." An important first step in advancing children's environmental health, therefore, is the documentation of chemical exposures across these the newly proposed lifestage exposome snapshots, which can be used as a starting point for improved children's health risk assessment.

Conclusion

Because the timing of exposure to toxicants during susceptible periods influences the health effects observed (U.S. EPA 2006, 2014), it is essential to introduce a lifestage framework into the concept of the exposome. The LEnS approach refines the original framework of the exposome to be more suitable for children's health by focusing on specific windows of susceptibility for target organ systems (Figure 4). LEnS analyses during critical lifestages have the potential to provide detailed information about co-exposures to chemicals with common mechanisms as well as information about the temporality of exposures during these key periods.

The LEnS approach also demonstrates the rationale and urgent need to take a broader view in risk assessment and regulation by considering cumulative exposures over critical periods of susceptibility for common target systems, rather than solely based on common mechanisms or chemical class (Figure 3). For example, information from LEnS analyses can be used to characterize an expanded OP risk cup that also considers exposures to other neurotoxicants. This approach is particularly important, given that OPs have been found to exert neurotoxicity through multiple mechanisms, including oxidative stress (U.S. EPA 2014). Without such improvements, children will be vulnerable to neurotoxicity from combinations of exposures to pesticides and other common chemicals. Continued childhood exposure to

neurotoxicants is not only personally detrimental but also collectively costly (Bellinger 2012; Bennett et al. 2016; Gould 2009; Trasande et al. 2005).

Information from LEnS analyses can also provide critical information to guide research and community-based public health efforts. Which co-exposures should be prioritized for toxicity testing—particularly to understand interaction effects? Which co-exposures are most relevant during different lifestages or seasons? How can these data guide effective intervention strategies (Thompson et al. 2008)?

Existing children's cohorts and related coordination efforts will aid in the application of the LEnS framework. The NIEHS Environmental Influences on Child Health Outcomes (ECHO) program and other birth cohorts provide the potential to obtain extensive data on exposome profiles during developmental windows. In addition, CHEAR provides important institutional support for children's health exposure analysis, which can improve our understanding of exposures during critical developmental periods (NIEHS 2016). To further facilitate the robust evaluation of exposures across the lifecourse, we echo previous calls for data sharing on a publicly accessible exposome database (Jones 2016; Teeguarden et al. 2016; Wild 2012). This repository would support efforts to combine exposomic analyses across different lifestages and populations, thereby providing a more complete representation of lifelong exposure patterns.

The adoption of the LEnS approach proposed here will improve the regulatory utility of the exposome by providing a framework for cumulative risk assessments during critical periods of development, thereby contributing to strengthened public health protection.

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